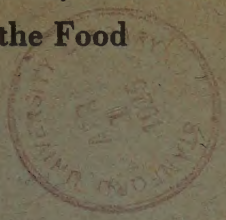
Quantitative Experiments Upon the Occurrence of Vitamin A in the Body, and the Influence of the Food



DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
THE FACULTY OF PURE SCIENCE IN
COLUMBIA UNIVERSITY

BY

LYMAN CROWELL BOYNTON

NEW YORK CITY

1924

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Dissertation

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Introduction

Through the inability of rats to grow on purified artificial food mixtures, seemingly adequate, and the resumption of growth with the addition of butter, or the ether extract of butter or of egg, McCollum and Davis (1), and Osborne and Mendel (2) demonstrated the existence of vitamin A in 1913. Failure on food lacking this did not occur at once, however, and it is the experience of workers in this field that normal young rats continue to grow for some time after being placed on diets presumably free of this factor. Such behavior pointed to the ability of the animal to store reserve supplies of vitamin A in his tissues (3, 4). Sherman, MacLeod, and Kramer (5) further pointed out that animals kept under like conditions upon the same diet deficient in vitamin A show different survival periods acording as their previous diet was rich or poor in this substance.

That certain glandular organs especially, were concerned in the storage function was early suggested by the discovery of the high potency of cod-liver oil by Osborne and Mendel (6), and the presence of large amounts of vitamin A in pig and beef livers (7, 8, 9, 10). The kidney of the pig also seems to be a rich source, while the brain and heart muscles of the same species seemed to contain less, though more than the skeletal muscle (7, 8, 11, 12).

Animal fats have been found to contain this vitamin, the concentration varying with the species, breed and with the source of the fat in the body. Beef fat contains considerable amounts (13, 14, 15). Drummond and Coward report similar amounts in the body fat of the dog and horse, less in that of sheep, and but small amounts in subcutaneous pig fat, though the perinephritic fat appears richer (16). Emmett and Lurose found the fat of the pancreas, thymus, and suprarenal glands to be deficient in vitamin A (17). Cod-testicle fat apparently contains large amounts (11).

That the diet of the animal may determine to a great degree the vitamin value of its tissues, has been demonstrated in the case of pig fat by Drummond et al (18). Likewise, milk contains vitamin A depending upon the amount contained in the food ingested by

the lactating female (19, 20, 21, 22, 23, 24, 25). Young fish apparently store this vitamin in their tissues in large amounts if given food containing ample quantities, but not otherwise (26).

The investigations of vitamin A content of animal tisues and products thus indicate the storage of this vitamin in the body, and suggest to some extent what tissues might be expected to function in this capacity. It is the purpose of this paper to present data of a more quantitative nature, obtained in a study of the distribution of vitamin A in the tissues of adult rats of known dietary histories.

Experimental

General Method.—The method used was based upon that suggested by Drummond and Coward (27), and Zilva and Miura (28). Young rats which had ceased to grow on a diet otherwise adequate, but free of vitamin A, had their diet supplemented by the tissues being tested, which served as the only sources of this vitamin. Resumption of maximum growth was not sought, but rather a comparative growth curve slightly above maintenance. The amount of vitamin A which allowed a growth of from about twenty to twenty-five grams during the experimental period of eight weeks was chosen as a standard for comparison. With such a rate of growth, it is possible to maintain the animal in a condition which is sufficiently sensitive to small changes in the vitamin A content of its food, while providing enough of the factor to prevent complete nutritive failure and death prior to the termination of the experimental period.

The Basal Diet.—The basal diet (Diet 701) used throughout the experiment was described by Kramer (29) who showed it to be free of vitamin A, but otherwise adequate. Normal growth with the production of young has been obtained with rats reared on this diet plus the addition of two per cent cod-liver oil. It (Diet 701) consists of:

Patent flour	82 per cent.
Meat residue (extracted)	10 per cent.
Salt mixture*	4 per cent.
Brewery yeast**	4 per cent.

*Salt mixture: Osborne and Mendel, *J. Biol. Chem.*, 37, 572, 1919).
**Yeast: Dry powdered brewers' yeast obtained from the Liberty Yeast Corporation, Newark, New Jersey.

The meat residue was extracted with about twice its weight of boiling ninety-five per cent alcohol under a reflux condenser for three periods of one hour each, the hot alcohol being filtered off by suction after each period.

Experimental Animals.—Young rats were put onto the fat-soluble vitamin-free basal diet at twenty-eight days of age, the time of weaning. When their body stores of this vitamin were

depleted as evidenced by cessation of growth and unmistakable signs of nutritive decline, usually though not always with the onset of xerophthalmia, they were put into separate round wire cages with raised screen bottoms and fed the tissues being tested. This preliminary period was as a rule of five or six weeks duration, and was terminated at a time when the rats were in as nearly the same physical condition as was possible to judge by loss of weight, gait, appearance of the coat and eyes, and freedom from respiratory troubles.

Assuming uniformity of the basal diet, differences in the ability of young rats to maintain on it alone would seem to depend largely upon the amounts of vitamin A stored in their bodies. Preliminary results on this point, with respect to the behavior of young rats of families fed on diets containing different amounts of milk in milk and whole wheat diets, have already been quoted by Sherman and Smith (30). The growth curves of rats from each of the following diets were reproduced. These rats, as well as others mentioned by Kramer (29) in survival period comparisons, are included in Table I.

Diet A contains 1/6 whole milk powder, 5/6 whole wheat;
Diet B contains 1/3 whole milk powder, 2/3 whole wheat;
Diet D contains 2/3 whole milk powder, 1/3 whole wheat.

TABLE I.

Showing the superior growth and longer length of life of 28-days-old rats, used as negative controls, when taken from diets containing greater percentage of milk and therefore of vitamin A, and placed on a basal diet free of this factor.

	Diet A		Diet B		Diet D	
	Gms.	No.	Gms.	No.	Gms.	No.
Av. weight at 28 days						
Males	32.8	(9)	43.8	(28)	50	(3)
Females	32.5	(11)	37.8	(9)	40	(3)
Both	32.6	(20)	42.8	(37)	45	(6)
Av. greatest gain recorded						
Males	15.2		50.1		63.0	
Females	21.2		38.7		47.3	
Both	18.5		48.2		55.2	
Av. Length of life on basal diet	days		days		days	
Males	31.9		64.2		66.3	
Females	40.4		63.7		69.1	
Both	36.5		64.1		67.8	

For the quantitative determinations herein reported, the experimental animals were therefore chosen from one diet, namely Diet B. A number of young from diets made up of the other combinations of milk and whole wheat, some from Diet B plus meat or almonds, or Diet B in which skim milk and butter fat were used in place of whole milk, have been used in some of the preliminary tests.

The initial weights of 279 Diet B rats at weaning were from 25 to 60 grams, usually lying between 30 and 55. The average initial weight for the 137 males was 41.5 grams and for the 142 females, 40.2 grams. The average gain as shown by the greatest weight

recorded during the preliminary period at the usual weekly weighings was 44.7 grams for the males and 36.5 for the females; the entire group making an average gain of 40.6 grams. For uniformity at the time of feeding the test portions, only those rats weighing from 60 to 95 grams were finally chosen for quantitative comparisons. Some animals below 60 grams, however, have been used where the others of the litter similarly treated were of like size.

The rats were weighed each week, at which time the food was weighed, and a record made of the amount eaten. Distilled water and the basal diet were always available.

Feeding Rats.—The rats whose tissues were used as sources of vitamin A, to study its distribution in the body, were young, healthy, vigorous adults reared on Diet B. Males were used for the most part; and in the few cases in which females were used, care was taken not to feed the tissues of rates whose recent history had been complicated by the bearing and suckling of young.

The animals were chloroformed as needed, the organs and other tissues to be tested, removed and kept in tightly closed glass containers in a refrigerator. Blood vessels were cut close to the organs, and excess blood removed from the larger vessels by gentle pressure and absorption on unglazed paper. When weighing out the small portions of moist tissue, care was taken to allow as nearly as possible the same amount of drainage and absorption in all cases. The parts were fed in small shallow glass dishes. Except in the case of the muscle and bone which were fed in amounts of a gram or more, the tissues were greedily devoured by the young rats. Muscle and bone were fed daily, while the other material as kidney, lung, and liver which were fed in much smaller amounts were fed on alternate days and the results stated in terms of average intake per day.

Distribution of Vitamin A in the Body of the Adult Rat.—Quantitative determinations were made of the relative vitamin A content of the liver, lung, kidney and muscle of the adult rat reared on a diet of one-third whole milk and two-thirds whole wheat plus 2% NaCl (Diet B). Indicative tests were also made on the long bones, brain, blood, body fat, heart, pancreas, spleen, and testes.

Young rats of the same litter were fed amounts of liver, lung, kidney and muscle, which were varied until those proportions were found which gave practically the same rate of growth, slightly above maintenance, over a period of eight weeks.

Muscle.—Fresh skeletal muscle from which the visible fat had been removed was fed in daily amounts of one, two, three and four grams (Tables II and VIII). Four grams daily was found necessary to insure enough vitamin A to maintain the rat during the experimental period and to permit some growth. The average

growth curve of seven rats receiving this amount of muscle will be seen in Fig. I to be slightly below those resulting from 0.1 gram respectively of kidney and lung. Feeding six grams of muscle per day was attempted, but a considerable amount remained uneaten.

Kidney.—The adhering fat was removed and the kidneys cut into cross-section portions for feeding. Tests were made feeding 0.5, 0.25, 0.10 and 0.075 gram daily (Tables III and VIII). The average growth of ten rats fed 0.10 gram per day as the source of vitamin A, in comparison with comparable amounts of muscle, lung, and liver, is given in Fig. I.

Lung—As will be seen from Fig. I, and Table IV, one-tenth gram of lung tissue daily provided sufficient vitamin A to permit some growth over a period of eight weeks. The average of nine tests gave results slightly above those obtained by feeding one-tenth gram of kidney, and a somewhat greater difference over four grams of muscle.

Not infrequently infected lungs were found in the adult rats used for feeding, even though they were apparently well nourished. Care was taken to remove any such infected areas and to feed only healthy tissue.

Liver.—In order to obtain growth curves corresponding to those of 4.0 grams of muscle and one tenth gram each of kidney and lung, different quantities of liver ranging from one gram to 0.01 gram per day were fed. Nine rats receiving one-tenth gram liver per day grew at a greater rate than was desired for this comparison. Twelve on 0.05 gram liver per day responded in a like degree. Reducing the portions fed to 0.02 gram, still resulted in uniformly better growth and condition than the amounts of other tissues being fed in comparison. Vitamin A available from only 0.01 gram daily, evidently is not sufficient, however, to insure growth or even maintenance over a period of eight weeks. Four of eight rats which were fed this small amount died before the expiration of the experimental period.

TABLE II.

Growth of Rats Receiving Four Grams Diet B Muscle.

	Lot No.	Weight weaning	Weight when fed tissue*	Weeks of Experiment: 1	2	3	4	5	6	7	8	Total gain in 8 weeks	Total food eaten in 8 weeks
				Weight in grams									
F	8210	50	93	94	101	98	109	104	111	118	118	25	385
M	8314	49	76	81	84	96	94	92	98	98	98	22	374
M	8761	47	94	91	94	97	102	106	107	101	109	15	364
M	8972	33	91	93	96	95	102	106	105	112	110	19	368
F	9110	47	91	88	90	94	94	94	95	97	102	11	361
F	9434	48	87	94	113	116	121	117	119	115	104	17	387
M	11551	35	72	76	83	85	84	85	87	84	90	18	312
Average (7)		44	86	88	94	97	101	101	103	104	104	18	364**

F—Female
M—Male

*Weight at time when the animal had ceased to grow on the basal ration and when the daily feeding of tissue to be tested was begun.

**By adding to this figure the approximate dry weight of muscle consumed (56 grams), the average total food intake for this group becomes 420 grams, which is comparable with that of those animals receiving lung, kidney, and liver in this comparison.

TABLE III.

Growth of Rats Receiving 0.1 gram Diet B Kidney

	Lot No.	Weight at weaning	Weight when fed tissue*	Weeks of Experiment 1	2	3	4	5	6	7	8	Total gain in 8 weeks	Total food eaten in 8 weeks
				Weight in grams									
F	7789	34	44	57	71	72	72	75	77	81	80	36	388
M	8115	47	78	82	90	96	100	106	107	105	113	35	502
F	8143	45	70	70	70	74	72	75	82	79	83	13	401
M	8210	50	91	101	100	105	110	108	114	117	121	30	475
F	8513	43	82	79	83	88	87	97	92	93	99	17	439
M	8761	47	82	82	87	84	82	85	86	87	92	10	386
M	8972	33	89	95	97	104	100	111	108	118	110	21	435
F	9110	47	81	78	86	85	86	80	84	82	88	7	392
M	9204	46	88	92	86	93	100	101	101	98	107	19	394
F	11551	35	60	58	65	70	76	83	84	81	84	16	362
Average (10)		44	77	79	83	87	89	92	94	94	98	21	417

TABLE IV.

Growth of Rats Receiving 0.1 gram Diet B Lung

	Lot No.	Weight at weaning	Weight when fed tissue*	Weeks of Experiment 1	2	3	4	5	6	7	8	Total gain in 8 weeks	Total food eaten in 8 weeks
				Weight in grams									
M	7789	37	69	66	76	86	97	108	113	119	118	47	413
M	8115	33	73	79	84	87	88	90	86	88	92	19	464
M	8210	50	91	100	106	108	115	111	119	121	128	37	468
M	8586	50	85	91	97	99	111	106	108	110	110	25	462
M	8761	42	72	73	72	72	71	79	82	82	88	16	389
F	9110	41	80	79	87	88	91	89	94	97	103	23	432
F	9204	33	87	93	102	108	108	108	115	118	126	39	466
F	9434	36	87	86	94	93	100	97	97	98	93	6	389
F	11551	40	60	64	66	71	70	72	67	71	69	9	319
Average (9)		40	78	81	87	90	95	96	98	100	103	25	426

TABLE V.

Growth of Rats Receiving 0.02 gram Diet B Liver

	Lot No.	Weight at weaning	Weight when fed tissue*	Weeks of Experiment 1	2	3	4	5	6	7	8	Total gain in 8 weeks	Total food eaten in 8 weeks
				Weight in grams									
F	8586	52	86	87	95	95	105	112	120	120	126	40	538
M	8761	38	72	73	76	76	86	85	88	95	101	29	430
M	8972	36	76	86	91	100	111	117	118	125	126	50	465
F	8976	46	84	92	100	107	114	115	124	125	130	46	517
M	9110	44	87	95	111	114	121	123	125	130	132	45	525
Average (5)		43	81	87	95	98	107	110	115	119	123	42	495

Growth of Rats Receiving 0.01 gram Diet B Liver

F	9687	45	85	83	94	102	104	103	104	108	109	24	421
F	11551	40	53	62	65	68	67	68	65	56	62	9	311
M	11644	39	81	92	93	91	81	88	73	See (1) below.			
M	11644	34	84	94	90	86	76	See (2) below.					
M	11664	33	80	81	82	82	79	78	82	79	72	—8	419
F	11664	42	83	90	81	84	88	86	89	90	92	9	370
F	11664	34	76	78	76	81	74	74	73	71	See (3) below.		
F	11664	26	67	73	74	78	69	62	See (4) below.				
Average (4)		40	75	79	81	84	85	84	85	83	84	9	380

(1) Died on the 43rd day of experiment.
(2) Died on the 28th day of experiment.
(3) Died on the 53rd day of experiment.
(4) Died on the 41st day of experiment.

*See footnote Table II.

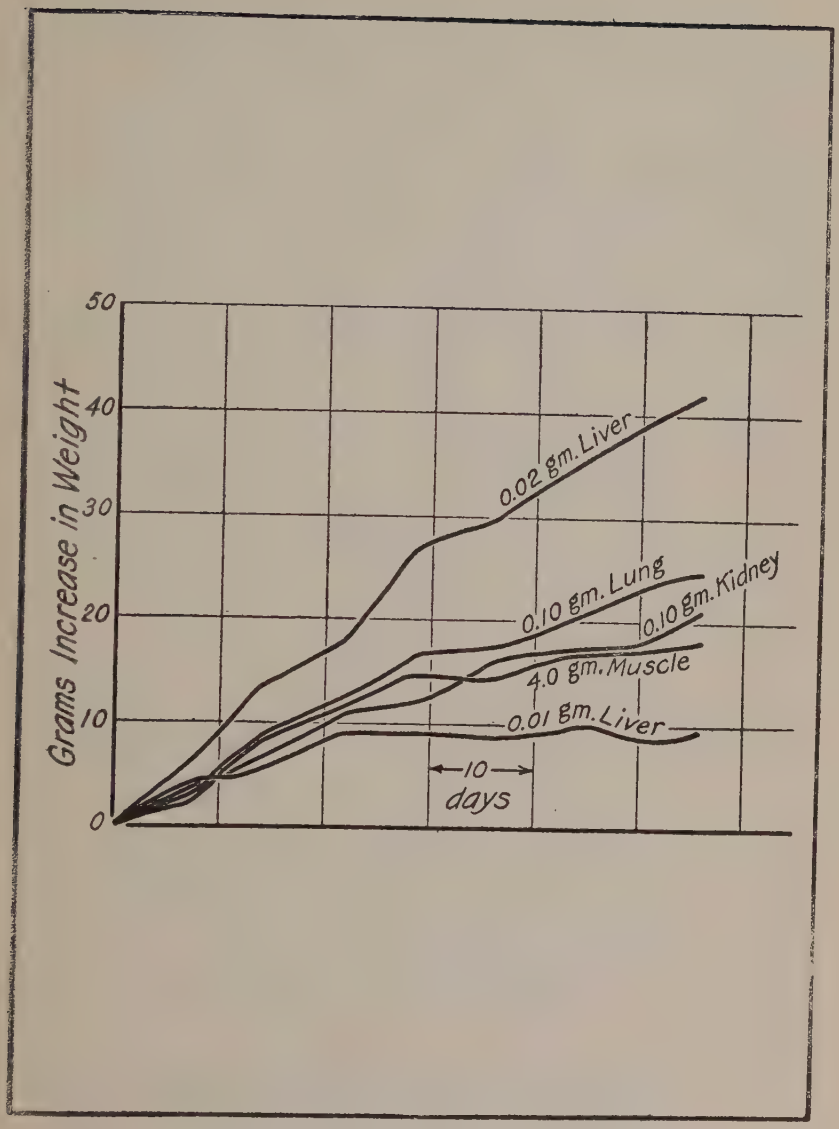

Fig I. Showing average growth curves of young rats which were fed tissues of Diet B adults as the sole source of vitamin A. The initial point of the curves represents the weight at the time of beginning the feeding of the tissues, after the animals had ceased to grow on a vitamin A-free basal diet.

An amount of liver between 0.01 and 0.02 gram then, furnishes as much of vitamin A as 4.0 grams of muscle, 0.1 gram of lung or 0.1 gram of kidney, as shown by the results obtained upon feeding such amounts of tissue (Fig. I).

While the above experiments were in progress, Coward, Lush, and Palmer (31) reported prompt curative results obtained by feeding as low as 0.1 gram per day of fresh livers of rats on rich vitamin A diets. One gram per day of livers of rats which had stopped growing on basal diets deficient in vitamin A gave good growth, while 0.7 gram resulted in failure.

Shortly afterward, Steenbock, Sell, and Nelson (32) described feeding dried rats' livers to the extent of 1% of the basal ration, which was on the border line of furnishing enough fat-soluble vitamin.

Goldblatt and Soames (33) have since published experiments in which fresh livers of rats receiving butter or cod-liver oil in their food were compared with the livers of rats which had ceased to grow on a diet deficient in the fat-soluble A, growth-promoting factor. 1.8 gram liver of the latter group gave definite growth in one case, but no growth was obtained with 0.5 or 1.0 gram per day. 0.25 gram liver of rats receiving 3% cod-liver oil afforded normal growth.

Other Tissues.—A number of other tissues were fed, for which it is not possible to present quantitative data, either due to lack of sufficient fresh material to maintain growth over the experimental period, or failure of the animals to eat, as was the case with bone (Table VI).

Bone.—The long bones and ribs were scraped free of adhering tissue, ground in a mortar to a paste and fed in one and two gram portions per day. In four cases in which the animals would eat the bone, there was some evidence of the presence of vitamin A, by prolonged life, and slight growth in the case of one rat which ate practically all of the two grams fed. Failure and death occurred, however, within a few days of the average life of negative controls on the basal diet alone. Weight for weight, there appears to be nearly as much vitamin A in the bone as in the muscle.

0.25 gram per day of brain, spleen, pancreas, or heart muscle failed to maintain or promote growth over the experimental period. One test was made of vitamin A in the testes from which all visible fat was removed, and the glands fed whole to the rat. Weight was maintained over a period of six weeks before death occurred on an amount averaging 0.5 gram per day, indicating the presence of vitamin A in rat testes, but in concentrations much lower than in kidney or in lung tissue.

The fat from two obese females* was removed, melted at a low temperature and filtered. Two rats receiving one half gram daily exhibited similar growth to those on one fifth this amount of liver per day.

Blood.—One test made with rats' blood points to the presence in it of considerable quantities of vitamin A. Anesthetized rats were bled, the blood collected in a glass-stoppered container and weighed. The clot was divided into several portions, transferred to shallow glass dishes, and administered to the rat on alternate days. By weighing the original container, the exact amount of blood fed was known. The rat was fed an amount averaging 0.65 gram fresh blood per day, and made a growth comparable to the rats of the same litter on 4.0 grams of muscle and 0.1 gram of kidney.

TABLE VI.

Miscellaneous Tests with Diet B. Tissues

Tissue	Amount per day	Lot No.	Results
Blood	0.65	8972 F	Grew 18 gms. in 9 weeks. Killed.
Bone	1 gm.	4274 M	Lived 25 days Control lived 3 days.
		4266 M	Grew 14 gms. Controls lived 18 and 22 days.
		5154 F	Lived 34 days. Control lived 19 days.
		5270 M	Lived 21 days. Control lived 22 days.
	2.0 gm	6164 M	Lived 25 days. Grew 11 grams.
Brain	0.25 gm.	6184 M	Lived 8 days.
		6184 F	Lived 11 days.
	0.56 gm.	8314 F	Lived 24 days. Control lived 14 days.
	0.51 gm.	9110 F	Lived 19 days. Control lived 29 days.
Fat	0.5 gm.	6143 M	Gained 62 gms. in 8 weeks. Killed.
		6143 M	Gained 50 gms. in 8 weeks. Killed.
Heart Muscle	0.25 gm.	6379 F	Lived 22 days. Control lived 11 days.
Pancreas	0.23 gm.	9434 F	Lived 34 days. Grew 11 gms. Control lived 18 days.
Spleen	0.25 gm.	6379 F	Lived 15 days. Control lived 11 days.
	0.23 gm.	8980 F	Lived 32 days. Control lived 32 days.
Testes	0.54 gm.	8314 M	Lived 46 days. Control lived 14 days.

Discussion.—From the above results obtained with tissues of adult Diet B rats, the kidney appears to be at least forty times as rich in vitamin A as the muscle, the lung more than forty times, and the liver, two hundred to four hundred times as rich in this vitamin as the muscle. The relative importance of these tissues as storage organs may be further emphasized by comparing the *total* quantities of vitamin A contained in each.

The following table represents approximately the distribution of this vitamin in a typical 275 gram rat. The weights assigned to the tissues are based on data of Donaldson (34), corroborated by weighing the organs of a group of Diet B rats such as were used for feeding. Values indicated by the smaller number of experiments with bone and blood are included. Further work, however, is necessary to fix their quantitiative relationship.

*It may be noted that abesity is very rare in this rat colony. Among over 10,000 rats less than 10 cases of obesity have been observed, and in these the obesity seemed to be associated with sterility.

Tissue	Approximate weight	Relative amounts of vitamin A per gram	Typical Distribution in organs tested (Skin, fat, etc. omitted)
Liver	12.80	50-100	90.0
Lungs	1.50	10.0	1.5
Kidneys	2.25	10.0	2.5
Blood	16.00	1.5	2.5
Muscles	120.00	0.25	3.0
Bones	15.00	0.25*	0.5

Influence of the Food

Relative Amounts of Vitamin A in Tissues of Diet B vs. Diet A rats. A group of adult rats reared on Diet B (one-third whole milk powder and two-thirds whole wheat) was selected for comparison with others of approximately the same age, many originally from the same litter, from Diet A (one-sixth whole milk powder and five-sixths whole wheat). Healthy adults approximately 8 to 10 months of age were used. The liver, muscle, lung and kidney were tested, tissues of each group of rats being fed to young animals of the same litter (Table VIII).

Liver.—That the liver of the rat receiving the greater amount of milk contains more of vitamin A was demonstrated in the ten direct comparisons made with it. It took 0.20 gram of Diet A liver to produce growth comparable with that resulting from the ingestion of only one-fourth as much, or 0.05 gram, Diet B liver in rats of the same litter.

Muscle.—In ten cases in which muscle was compared, there was evidence of more vitamin A in the Diet B rat muscle, but the difference is not nearly so large as in the case of liver. There appears to be about a third more in Diet B than in Diet A muscle.

Kidney.—No difference in the concentration of vitamin A in the kidneys of the Diet B and Diet A rats could be detected in three comparisons.

Lung.—In both comparisons of Diet B and Diet A lung, better growth was obtained with the lung of those rats receiving the larger proportion of milk in their diets. Rat No. 7726 on 0.05 gram Diet B lung grew more than No. 7727 of the same litter on 0.10 gram Diet A lung, and rat No. 7791, also receiving 0.10 gram of Diet A lung, grew at the rate of about half that of No. 7789 on the same amount of Diet B lung. Several writers have called attention to the susceptibility of animals whose diets are deficient in vitamin A to respiratory diseases (15, 30, 32, 35). This is borne out by the experience of this laboratory which shows a greater incidence of lung disease among the rats on Diet A than among those on Diet B. That the lung tissue itself of animals reared on diets relatively poor in this vitamin should actually contain less than that of those receiving more ample amounts of vitamin A, then becomes especially significant. Further evidence that an increased vitamin A concentration in the

lung tissue results from a high intake of this vitamin was obtained in the experiments below, with the additions of cod-liver oil to the basal diet.

TABLE VIII

Comparison of Growth of Young Rats Fed Tissues of Diet A and Diet B Adults as Sources of Vitamin A

		Fed Diet A Tissues				Fed Diet B Tissues				
	Lot No.	Tissue	Amt. fed per day	Weight when fed tissue	Total gain in 8 weeks	Lot No.	Tissue	Amt. fed per day	Weight when fed tissue	Total gain in 8 weeks
F	5457	Liver	0.05	73		F 5457	Liver	0.05	70	48
			Lived 8 days							
F	6015	"	0.05	67						
			Lived 31 days							
F	6164	"	0.05	60						
			Lived 18 days							
F	7789	"	0.10	49	23	F 7789	"	0.10	50	45
F	7789	"	0.15	48	17					
F	7789	"	0.20	54	18					
M	7831	"	0.10	66	26	M 7831	"	0.05	76	63
M	7831	"	0.20	64	40					
F	7961	"	0.10	85	14	F 7961	"	0.05	83	49
M	8067	"	0.20	72	49	M 8067	"	0.05	67	73
M	4676	Muscle	1.00	117		M 4676	Muscle	1.00	113	
			Lived 25 days						Lived 25 days	
M	4683	"	1.00	88		M 4683	"	1.00	93	
			Lived 24 days						Lived 21 days	
F	4676	"	2.00	88		F 4676	"	2.00	83	
			Lived 10 days						Lived 38 days	
F	4683	"	2.00	82		F 4683	"	2.00	80	
			Lived 24 days						Lived 34 days	
F	5457	"	3.00	80		F 5457	"	3.00	70	
			Lived 14 days						Lived 35 days	
M	5457	"	3.00	69						
			Lived 19 days							
M	6015	"	3.00	69		M 6015	"	3.00	66	22
			Lived 42 days							
F	6164	"	3.00	56		F 6164	"	3.00	67	
			Lived 27 days						Lived 47 days	
F	7628	"	3.00	57		F 7628	"	3.00	62	16
			Lived 49 days							
F	7628	"	4.00	60	15					
						M 5156	"	3.00	101	23
						M 7608	"	3.00	72	13
						F 7831	Kidney	0.075	65	19
						F 8124	"	0.075	87	15
M	7724	Kidney	0.15	74	56	M 7724	"	0.075	81	17
F	7789	"	0.10	41	41	F 7789	"	0.10	44	36
						F 6184	"	0.10	46	27
M	6184	"	0.25	50	80	M 6184	"	0.25	54	86
M	7724	Lung	0.10	70	19	M 7724	Lung	0.05	64	25
M	7789	"	0.10	56	30	M 7789	"	0.10	69	50

Experiments with ten-weeks old rats receiving cod-liver oil additions to the basal diet. The tissues of three groups of ten-weeks old rats were tested for vitamin A. Their respective diets since weaning, six weeks previous, had been the vitamin A-free basal diet alone, the basal diet supplemented with one percent, and with two percent cod-liver oil. A fresh supply of ten-weeks old rat tissues was maintained by placing rats from the same litter on each of the three diets each week. During the sixth week afterward, they were killed and their tissues fed to test animals.

Amounts were fed which in the case of adult Diet B rat tissues had been more than enough for maintenance—4.0 grams muscle, 0.1 gram kidney, 0.1 gram lung, and 0.01 gram liver per day (Table IX). In no case was there any appreciable difference between the growth of those rats which were fed the tissues of rats receiving no cod-liver oil, and the negative controls. Ten milligrams of liver from a ten-weeks old Diet B rat showed an appreciable amount of vitamin A, but not enough for maintenance. The same amount from the rats receiving one percent cod-liver oil in their diets gave maintenance in one instance, while this amount of liver from those receiving two percent cod-liver oil showed more growth, about equal to 0.02 gram liver of an adult Diet B rat.

TABLE IX

Tests of Tissues of Ten-Weeks Old Rats Receiving Cod-Liver Oil

Tissue Fed	Lot No.	Weight at weaning	Weight when fed tissues	Total gain in 8 weeks	Total food eaten in 8 weeks
Muscle 4.0 grams					
Rat on basal diet ..... M	10991	43	81	Lived 20 days	
				Control lived 24 days	
do. plus 1% c.l.o. (1) ..... F	10991	43	82	Lived 21 days	
M	10991	45	89	6*	340
do. plus 2% c.l.o. ..... M	10991	40	92	6**	353
F	10991	38	78	23	369
Kidney 0.1 gram					
Rat on basal diet ..... M	11028	34	68	Lived 14 days	
				Control lived 9 days	
M	11132	55	72	Lived 26 days	
				Control lived 20 days	
do plus 1% c.l.o. ..... M	11028	30	69	80	508
F	11132	53	80	61	475
do. plus 2% c.l.o. ..... M	11028	32	68	62	476
F	11132	57	84	60	500
Lung 0.1 gram					
Rat on basal diet ..... F	11061	45	78	Lived 42 days	
				Control lived 20 days	
F	11069	40	82	Lived 13 days	
				Control lived 11 days	
do. plus 1% c.l.o. ..... F	11061	43	79	61	521
F	11069	43	74	48	404
do. plus 2% c.l.o. ..... F	11061	47	83	66	505
F	11069	35	73	68	473
Liver 0.01 gram					
Rat on basal diet ..... F	10902	47	85	Lived 10 days	
				Control lived 32 days	
F	10902	44	79	Lived 26 days	
do. plus 1% c.l.o. ..... F	10902	41	79	Lived 31 days	
F	10902	45	76	1	339
do. plus 2% c.l.o. ..... F	10902	44	82	33	417
F	10902	44	75	32	417

*Gained 15 grams in first four weeks.
**Gained 23 grams in first five weeks.
(1) c.l.o.—cod-liver oil.

In the case of muscle of rats receiving two percent cod-liver oil, however, the growth made on 4.0 grams per day was no better than from that amount of Diet B rats' muscle. As long as there is a liberal supply in the food, the vitamin A content of the muscle appears to maintain a more constant value than is the case with the liver, lung or kidney.

Both 0.1 gram lung and 0.1 gram kidney of rats receiving cod-liver oil resulted in a rate of growth at least twice that from the same amount of these tissues from Diet B rats. At this level of growth, it was not possible to detect a great amount of difference between that produced by the tissues of the rats receiving one percent and two percent of the oil. Both tests of the lung, however, from rats of the richer diet resulted in the greater growth.

Such experiments comparing tissues of rats receiving cod-liver oil additions to the basal diet, with those of rats denied such supplements, confirm the hypothesis implied in the comparison of rats from Diets B and A: namely, the difference in amounts of vitamin A in their tissues, particularly of this vitamin stored in the liver and present in the lung, are directly attributable to the different amounts of vitamin A received in their food.

Summary.

1. The distribution of vitamin A in the body of the rat was studied by feeding the tissues of adult animals, as the sole source of this vitamin to young rats which had ceased to grow on a diet otherwise adequate.

2. An average of all directly comparable results on adult rats reared on a diet of one-third whole milk powder and two-thirds whole wheat, plus 2% NaCl, shows the kidney to be at least forty times as rich as the muscle; lung more than forty times; and liver from two hundred to four hundred times as rich in vitamin A per gram as muscle. There is apparently nearly as much of this vitamin per gram of bone as of muscle, while one test with blood showed about one-sixth that of kidney.

3. Animals whose food contains one-third whole milk powder and two-thirds whole wheat (Diet B) were shown to store more vitamin A in their bodies than adults of approximately the same age on a diet containing but one-sixth whole milk powder (Diet A). Diet B young placed at weaning on a basal diet free of vitamin A, but otherwise adequate grow more and live longer than Diet A rats under the same conditions. Tissues of adult Diet B rats actually contain more vitamin A than those of Diet A. The liver appears to be about four times as rich, the lung

twice as rich, and the muscle about a third richer than the corresponding Diet A tissues.

4. That the difference in the amount of vitamin A found in the tissues is directly attributable to the different amounts of it in the food is shown by a comparison of the growth-promoting power of tissues of ten-weeks old rats receiving cod-liver oil additions to the basal diet, with that of tissues of rats of the same age and diet without cod-liver oil. Of the latter group, the muscle, lung, kidney and liver were fed in the same amounts which in the case of tissues of Diet B rats had proven more than sufficient for maintenance, and in no case was there any appreciable difference from the negative controls. Better growth was obtained from the lung, kidney and liver of the rats receiving cod-liver oil, than from the same amounts of tissues of Diet B rats of the same age. That more vitamin A is contained in the muscle of such rats, however, is not apparent.

Vita.

Lyman Crowell Boynton was born in Ashtabula, Ohio, November 4, 1893. He attended Denison University, Granville, Ohio from 1912 to 1914, and the University of Michigan from 1914 to 1916, where he received the degree of Bachelor of Science. During the war, he served a year in the Chemical Warfare Service, United States Army.

He was a graduate student at the University of California, 1920-21. In 1921, he was appointed Research Assistant in Food Chemistry, Columbia University, which position he now holds. He received the degree of Master of Arts in 1922. He has been a graduate student in the Faculty of Pure Science since 1921, except during the autumn quarter of 1923, when he was acting instructor in biochemistry, Stanford University, California.

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